1993

# Bovine Embryos from Bluetongue Infected Donors did not Transmit Virus to Susceptible Recipients

John A. Acree
*APHIS*

Sherrill E. Echternkamp
*U.S. Meat Animal Research Center*

Steve M. Kappes
*U.S. Meat Animal Research Center*

Gary S. Ross
*Arthropod-borne Animal Disease Research Lab*

Albert J. Luedke
*Arthropod-borne Animal Disease Research Lab*

*See next page for additional authors*

# Bovine Embryos from Bluetongue Infected Donors did not Transmit Virus to Susceptible Recipients

John A. Acree, Sherrill E. Echternkamp, Steve M. Kappes, Gary S. Ross, Albert J. Luedke, and James E. Pearson[1]

## Introduction

The recent development of methodology for the successful cryopreservation (i.e., ultra low temperature freezing) of bovine embryos has expanded the opportunities for international movement of bovine embryos and has facilitated the exchange of germplasm within and among countries. Unfortunately, frozen embryos also provide an excellent vehicle for the distribution of pathogenic agents (e.g., bluetongue virus, foot and mouth disease, etc.) between livestock populations and between countries. Most pathogens that are on the surface of an embryo with an intact zona pellucida (a translucent mucopolysaccharide shell surrounding the embryo) can be removed by washing. The washing procedure consists of subjecting the embryos to 10 to 12 changes of medium, each change of medium resulting in a 100-fold dilution of the medium. However, some viruses (e.g., infectious bovine rhinotracheitis virus and vesicular stomatitis virus) adhere firmly to the zona pellucida and are not removed by the 10 to 12 changes of medium. The objective of this study was to determine the risk of transmitting the bluetongue virus by washed bovine embryos from infected cows. The present study was conducted with a sufficient number of animals to estimate the probability of bluetongue virus transmission by the transfer of embryos from an infected donor, to mimic as closely as possible the natural infection of embryo donors, and to collect the embryos at various periods during the donors' interaction with the virus.

## Procedure

Healthy, nonpregnant, nonlactating, BTV-susceptible, bovine heifers (n=59) were infected with BTV serotype 11 strain C075B300 (BTV-11) by bites from infected *Culicoides variipennis* ssp. *sonorensis*. The midges (gnats), in lots of 25 to 100, were given access through a nylon membrane to a shaved area on the back of the donor heifers for 30 min. In addition, the heifers were inoculated intradermally with a suspension of homogenized infected *C. variipennis sonorensis* at the end of the midge exposure. Virus isolation and antibody titers confirmed that all 59 heifers became infected with BTV-11. *C. variipennis* is a biting midge and the principal vector of BTV in North America. The midges used in this study came from the AK colony at the Arthropod-borne Animal Disease Research Laboratory at Laramie, Wyoming.

Between 5 and 8 days after infection, the 59 viremic donor heifers were given twice daily injections of FSH for 4 days (total dosage of 34 mg) to initiate superovulation and 35 mg of prostaglandin $F_{2\alpha}$ on the last day of FSH treatment to induce estrus. Heifers were artificially inseminated at 12, 24, and 36 hr after onset of estrus (day 0) with semen from bulls that were negative for BTV. Embryos were collected nonsurgically on day 7 or 8. Isolation of bluetongue virus and elevated antibody titers in blood samples indicated that all 59 donors were viremic at embryo collection (i.e., "acute" donors). At 1.5 and 3 mo after infection, a second and third collection of embryos from these donors were attempted. Blood taken at the time of embryo collection contained antibodies to BTV-11 but no viruses were isolated so the animals were considered to be "convalescent" donors.

At all three embryo collections, the embryos were washed 10 times in 2 ml aliquots of fresh sterile medium. Embryos were agitated throughout the wash volume, and a new pipet was used to carry them to the next wash in 20 µl of fluid. All embryos (up to 10) from a single donor were washed together. Washed embryos were examined microscopically at 50X magnification. Only grade 1 and 2 morulae and blastocysts with an apparently intact zona pellucida were selected for transfer into BTV negative recipient females. Each selected embryo was identified to its dam and sire and was transferred nonsurgically into a recipient either within a few hr after collection or frozen for later transfer. Embryos unacceptable for transfer were frozen separately for each donor, and virus isolation was attempted later at the National Veterinary Services Laboratory, Ames, Iowa.

After receiving embryos, the recipients were observed for signs of disease and periodically tested for BTV group specific antibody. If the recipient did not become pregnant to the embryo transfer but remained free of signs of, or antibody to, BTV for at least 60 days after receiving the embryo, it was assumed that there had been no lateral transmission of virus from infected donor to recipient via the embryo. If the recipient became pregnant but remained free of signs of, or antibody to, BTV for at least 60 days after abortion or parturition, it was assumed that there had been no transplacental exposure to a viremic fetus that had been infected vertically via the embryo. If the offspring remained free of signs of, or antibody to, BTV for at least 60 days after its birth, it was assumed that there had been no vertical transmission of virus from infected donor to offspring via the embryo. Pregnant recipients were housed at the U.S. Dairy Forage Research Center, Prairie du Sac, Wisconsin, (i.e., a BTV-free area) from May 4 to October 28, the vector season for *C. variipennis* in Nebraska.

## Results

A total of 169 embryos were collected from 34 of 59 viremic donors (Table 1). Virus was not isolated from the 57 nontransferable embryos examined. Recipients of 108 fresh and 2 thawed transferable embryos remained free of evidence of BTV infection for more than 60 days after embryo transfer. The 36 calves (Table 1) resulting from these embryo transfers had no evidence of BTV infection at 60+ days of age and their surrogate dams were also free of signs of BTV infection at that time; two embryos were not used.

A total of 141 embryos (Table 1) were collected from 44 convalescent donors (i.e., second and third embryo collection at 1.5 to 3 mo, respectively, after BTV infection). Virus was not isolated from blood samples taken from the donors at the time of embryo collection or from 20 washed embryos submitted for virus isolation. Recipients of 59 fresh and 62 thawed embryos remained free of evidence of BTV infection

[1]Acree is a veterinary epidemiologist, Program Planning Staff, APHIS, Veterinary Services, Hyattsville, MD; Echternkamp is a research physiologist, Reproduction Research Unit, MARC; Kappes is a research physiologist, Genetics and Breeding Research Unit, MARC; Ross is herd health veterinarian, Luedke is a research veterinary medical officer, Arthropod-borne Animal Disease Research Lab, Laramie, WY; Pearson is chief, Diagnostic Virology, National Veterinary Services Laboratory, Ames, IA.

for more than 60 days after embryo transfer. The 52 calves (Table 1) resulting from these transfers had no evidence of BTV infection at 60+ days of age; their surrogate dams also remained free of BTV infection. Furthermore, no BTV virus was isolated from the flush fluids or wash fluids. Failure to isolate BTV virus from flush fluids of acute or convalescent donors may have been because very few of the embryo collections contained appreciable amounts of blood. In summary, we found no evidence of BTV-11 transmission from viremic or convalescent donors to susceptible recipients or their offspring by 7- to 8-day embryos that had been washed according to the recommendations of the International Embryo Transfer Society.

**Table 1—Incidence of bluetongue virus (BTV) transmission**

| Type of donor | Donors | Embryos | | Recipient | | Calves |
|---|---|---|---|---|---|---|
| | | Transferable | Nontransferable | Pregnant | Nonpregnant | |
| Acute: | | | | | | |
| Total no. | 59 (34[a]) | 112 | 57 | 36 | 74 | 36 |
| No. with BTV | 59 | 0 | 0 | 0 | 0 | 0 |
| No. seropositive | 59 | — | — | 0 | 0 | 0 |
| Convalescent: | | | | | | |
| Total no. | 59 (44[a]) | 121 | 20 | 52 | 69 | 52 |
| No. with BTV | 0 | 0 | 0 | 0 | 0 | 0 |
| No. seropositive | 59 | — | — | 0 | 0 | 0 |

[a] Number of donors contributing embryos are in parentheses.